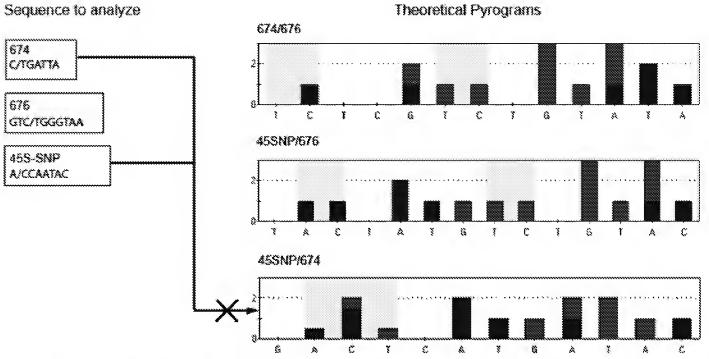

Fig. 1. Compatibility of extension reactions for multiplexing is shown. The "sequence to analyze" and theoretical multiplexing pattern are determined by assay design software 1.0 and PSQ HS 96A 1.2 software, respectively. Black and gray bars represent expected contribution from each of the primer reactions. Areas of shading represent polymorphic sites to be interrogated. The "X" sign indicates the incompatibility of two templates for multiplexing as the theoretical pyrograms show overlapping bars at the polymorphic site.

3.1. Multiplex Assay Design Scheme

The essential step in multiplex assay design is to find an optimal nucleotide dispensation order capable of discriminating multiple nucleotide incorporation patterns in the same run. Specifically, the peaks from each polymorphic and at least one nonpolymorphic sites of the same primer should not overlap with the peaks from the other extension reactions *(19)*. Other peaks in the multiplex incorporation pattern may be composite peaks. This requirement, however, may not allow certain "sequences to analyze" to be combined for optimal design. **Figure 1** shows the optimal dispensation orders and theoretical Pyrogram charts obtained for the combination of 674/676 and 45SNP/676, but not 45SNP/674. The general scheme of finding an optimal dispensation order is essentially iterative as outlined in **Fig. 2**.

EXHIBIT A

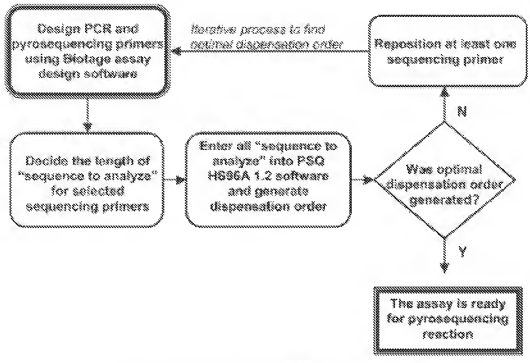

Fig. 2. Multiplex Pyrosequencing® assay design process.

EXHIBIT A